510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number:
k040111

B. Analyte:
Myeloperoxidase (MPO), Proteinase 3 (PR3)

C. Type of Test:
Flow Cytometry-based, homogeneous, multiplexed, microparticle fluorescent immunoassay, semi-quantitative

D. Applicant:
Zeus Scientific, Inc.

E. Proprietary and Established Names:
Zeus Scientific, Inc. AtheNA Multi-Lyte™ MPO/PR3 IgG Test System

F. Regulatory Information:

1. Regulation section:
   21 CFR §866.5660 Multiple Autoantibodies Immunological Test System
2. Classification:
   Class II
3. Product Code:
   MOB, Anti-neutrophil cytoplasmic antibody (ANCA) immunological test system
4. Panel:
   IM 82

G. Intended Use:

1. Intended use(s):
   The Zeus Scientific, Inc. AtheNA Multi-Lyte™ MPO/PR3 IgG Test System is intended for the qualitative and/or semi-quantitative detection of IgG class antibody to two separate ANCA Antigens (Myeloperoxidase and Proteinase 3) in human serum. The test system is intended to be used as an aid in the diagnosis of various autoimmune vasculitic disorders characterized by elevated levels of anti-neutrophil cytoplasmic antibodies (ANCA). MPO and/or PR3 may be associated with autoimmune disorders such as Wegener's Granulomatosis, ICGN, MPA and PRS. This test is for *in vitro* diagnostic use.
2. Indication(s) for use:
   The Zeus Scientific, Inc. AtheNA Multi-Lyte™ MPO/PR3 IgG Test System is intended for the qualitative and/or semi-quantitative detection of IgG

autoantibodies to human myeloperoxidase (MPO) and/or human proteinase 3 (PR3) in human serum. The results of this serological test together with other clinical findings may aid in the diagnosis of systemic vasculitides (SV). This test is for *in vitro* diagnostic use.

3. Special condition for use statement(s):
   The device is for prescription use only.
4. Special instrument Requirements:
   AtheNA Multi-Lyte instrument

H. Device Description:

The AtheNA Multi-Lyte MPO/PR3 IgG assay consists of:

- multiplexed bead suspension containing 5.6 micron polystyrene beads conjugated with human MPO or PR3. The bead mix also contains one bead set designed to detect non-specific antibodies in the patient sample (if present) and four separate bead sets used for assay calibration;
- phycoerythrin conjugated goat anti-human IgG (γ-chain specific);
- human positive and negative serum controls;
- sample diluent;
- 96-well sample dilution plate;
- 96-well filtration plate; and
- 96-well assay plate.

All these reagents are to be used on the AtheNA Multi-Lyte instrument.

I. Substantial Equivalence Information:

1. Predicate device name(s):
   Zeus Scientific, Inc. MPO and PR3 IgG ELISA Test Systems
2. Predicate K number(s):
   k964446, k964448
3. Comparison with predicate:

AtheNA Multi-Lyte MPO/PR3	MPO and PR3 ELISA assays
A. Similarities	
Intended Use. - For the qualitative and/or semi-quantitative detection of IgG autoantibodies to human myeloperoxidase (MPO) and/or human proteinase 3 (PR3) in human serum as an aid in the diagnosis of systemic vasculitides (SV) such as Wegener's Granulomatosis, ICGN, MPA and PRS.	Same
Sample Type – Serum	Same
Conjugate – Polyclonal goat anti-human IgG	Same
B. Differences	
Assay Method – Flow cytometry based	ELISA

AtheNA Multi-Lyte MPO/PR3	MPO and PR3 ELISA assays
Assay Format - Multiplexed	Individual analytes
Solid Phase – Differentially colored, carboxylated microspheres	Microtiter well
Conjugate label - Phycoerythrin	HR peroxidase
Measurement - Fluorescence	Optical density
Instruments – AtheNA Multi-Lyte analyzer	ELISA reader
Dynamic Range – 0 to 2451 AU/mL (MPO) 0 to 3405 AU/mL (PR3)	Not furnished

J. Standard/Guidance Document Referenced (if applicable):

None referenced

K. Test Principle:

The Zeus Scientific, Inc. AtheNA Multi-Lyte™ MPO/PR3 IgG Test System is a multiplexed, fluorescent immunoassay performed on the AtheNA Multi-Lyte instrument. Each of the antigens, assay control and calibrator are coupled to different colored polystyrene microspheres. Diluted patient serum (1:21) is mixed with the bead suspension in microtiter well and incubated. If specific antibodies are present, they will bind to the immobilized antigens on one or more of the bead sets. The microspheres are rinsed to remove non-reactive serum proteins. Then β-phycoerytherin (PE)-conjugated goat anti-human IgG (Fc specific) is added to each well and incubated. The conjugate will react with IgG antibody immobilized on the microspheres. The bead suspension is analyzed by the AtheNA Multi-Lyte instrument. The bead sets are identified and the amount of PE conjugate (fluorescence) is determined for each bead set. Using the Intra-Well Calibration Technology, internal calibration bead sets are used to convert the raw fluorescence into units.

The test principle and performance of the microparticle-based immunoassay (flow-cytometry) for the AtheNA Multi-Lyte™ instrument was supported in k011244 and k021103.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   To evaluate both intra-assay and inter-assay reproducibility, six specimens were tested. These samples were selected to include two negative, two strong positive and two weakly positive or near the cut-off of the assay. On each day of testing, two diluted aliquots of each sample were assayed in four replicates. The procedure was followed for three days.

   Intra-assay

	Strong Positive	Weak Positive	Negative
MPO (%CV)	4.6% - 9.7%	4.5% - 11%	11.7% - 38.2%
PR3 (%CV)	3.2% - 9.7%	6.4% - 12.8%	7.6% - 33.5%

Inter-assay

	Strong Positive	Weak Positive	Negative
MPO (%CV)	8.0% - 11.8%	9.1% - 13.2%	13.9% - 33.1%
PR3 (%CV)	3.2% - 7.8%	9.4% - 10.3%	9.3% - 26.7%

b. Linearity/assay reportable range:
Dynamic ranges for MPO and PR3 are 0 – 2451 AU/mL and 0 - 3405 AU/mL respectively. It should be noted that the amount of fluorescence is related to the quantity of autoantibody present on the bead but in a non-linear fashion. The antibody concentrations correspond to fluorescent signal changes but are not directly proportional.

c. Traceability (controls, calibrators, or method):
No reference standards or method available. For each assay kit, a negative and a positive control are included.

d. Detection limit:
Not relevant for this assay.

e. Analytical specificity:
To assess potential cross-reactivity to other antibodies, 15 serum samples consisted of 2 EBV positive, 3 RA, 7 ANA, and 3 TORCH positive were analyzed. Cross-reactivity was observed with one ANA sample for both MPO and PR3.
Interference from serum components was evaluated by testing twenty MPO/PR3 negative serum samples consisting of 5 hemolyzed, 5 high triglyceride, 5 high bilirubin and 5 high IgG. Of the 20 samples, one hemolyzed sample and one with high bilirubin were positive for both MPO and PR3.

f. Assay cut-off:
The cut-off value was determined using 150 normal blood donor samples and arbitrarily set at mean fluorescence plus 3 standard deviations (SD). For MPO, 140 of the 150 samples were negative with a mean fluorescence of 31 and a SD of 20.96. The cut-off value for MPO is 93.9. For PR3, 117 of the 150 samples were negative with a mean fluorescence of 45 and a SD of 26.96. The cut-off value for PR3 is 125.5.

2. Comparison studies:

a. Method comparison with predicate device:
There were a total of 445 specimens tested in the comparative study. Samples were categorized according to the following: normal blood donors (n=150), specimens sent to a serology laboratory for routine MPO and PR3 testing (n=122) and specimens obtained from patients diagnosed with disorders associated with MPO and/or PR3 (n=173 with 74 MPO, 67 PR3 and 32 MPO or PR3). Results are summarized in tables below.
MPO

		ELISA			
		Positive	Negative	Equivocal*	Total
AtheNA	Positive	55	39	2	96
	Negative	2	338	1	341
	Equivocal*	1	4	0	5
	Invalid**	1	2	0	3
	Total	59	383	3	445

*Excluded from calculations

**Three samples were invalid by AtheNA and excluded from calculations.

Positive agreement = 55/57 = 96.5%
Negative agreement = 338/377 = 89.6%
Total agreement = 393/434 = 90.6%

PR3

		ELISA			
		Positive	Negative	Equivocal*	Total
AtheNA	Positive	85	53	1	139
	Negative	6	283	0	289
	Equivocal*	4	10	0	14
	Invalid**	1	2	0	3
	Total	96	348	1	445

*Excluded from calculations

**Three samples were invalid by AtheNA and excluded from calculations.

Positive agreement = 85/91 = 93.4%
Negative agreement = 283/336 = 84.7%
Total agreement = 368/427 = 86.2%

b. Matrix comparison:

Serum is the only recommended matrix for both assays.

3. Clinical studies:

a. Clinical sensitivity:

MPO

Clinical sensitivity was evaluated using 74 clinically defined serum samples from patients diagnosed with autoimmune systemic vasculides. Of the 74 samples tested, 45/74 (60.8%) were positive, 2/74 (2.7%) were equivocal and 27/74 (36.5%) were negative. The clinical sensitivity of the AtheNA Multi-Lyte MPO IgG test system was therefore determined to be 60.8% (95% CI 49.7% to 71.9%).

PR3

Clinical sensitivity was evaluated using 67 clinically defined serum samples from patients diagnosed with autoimmune systemic vasculides. Of the 67 samples tested, 37/67 (55.2%) were positive, 2/67 (3.0%) were equivocal and 28/67 (47.8%) were negative. The clinical sensitivity of the AtheNA Multi-Lyte PR3 IgG test system was therefore determined to be 55.2% (95% CI 43.3% to 67.1%).

b. Clinical specificity:

MPO

Clinical specificity was evaluated using 150 normal blood donors presumably free of autoimmune disease. Of the 150 specimens tested, 9/150 (6%) were positive, 1/150 (0.7%) were equivocal and 140/150 (93.3%) were negative. The clinical specificity for the AtheNA Multi-Lyte MPO IgG test system was therefore determined to be 93.3% (95% CI 89.3% to 97.3%).

PR3

Clinical specificity was evaluated using 150 normal blood donors presumably free of autoimmune disease. Of the 150 specimens tested, 27/150 (18%) were positive, 6/150 (4%) were equivocal and 117/150 (78%) were negative. The clinical specificity for the AtheNA Multi-Lyte PR3 IgG test system was therefore determined to be 78% (95% CI 71.4% to 84.63%).

c. Other clinical supportive data (when a and b are not applicable)
Not applicable.

4. Clinical cut-off:
   See assay cut-off.
5. Expected values/Reference range:
   The expected value in the normal population is negative. Of the 150 normal donor samples, 6% were positive for anti-MPO and 18% for anti-PR3 IgG autoantibodies. Of the 173 clinically defined patient samples, 60.1% were positive for anti-MPO and anti-PR3 IgG autoantibodies.

M. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.